

What is claimed is:

- 1           1.    A method for formulating an enzyme comprising:  
2               obtaining at least one glucose oxidase gene;  
3               creating at least one mutated glucose oxidase gene;  
4               introducing each mutated glucose oxidase gene into separate expression vectors;  
5               inserting the expression vectors into host organisms;  
6               growing colonies of the host organisms; and  
7               screening the colonies for desirable properties.
- 1           2.    A method for formulating an enzyme according to claim 1, wherein screening the  
2 colonies for desirable properties comprises:  
3               determining whether the colonies contain active glucose oxidase; and  
4               determining whether the colonies have peroxide resistant properties.
- 1           3.    A method for formulating an enzyme according to claim 2, wherein screening the  
2 colonies for desirable properties further comprises testing glucose oxidase from the colonies for  
3 functionality.
- 1           4.    A method for formulating an enzyme according to claim 2, wherein determining  
2 whether the colonies have peroxide resistant properties is only performed if results of  
3 determining whether the colonies contain active glucose oxidase are positive.
- 1           5.    A method for formulating an enzyme according to claim 3, wherein testing  
2 glucose oxidase from the colonies for functionality is only performed if results of determining

3 whether the colonies contain active glucose oxidase are positive and if results of determining  
4 whether the colonies have peroxide resistant properties are positive.

1 6. A method for formulating an enzyme according to claim 2, wherein determining  
2 whether the colonies have active glucose oxidase comprises employing a substance that changes  
3 color in the presence of active glucose oxidase.

1 7. A method for formulating an enzyme according to claim 6, wherein the substance  
2 is leuco-crystal-violet.

1 8. A method for formulating an enzyme according to claim 2, wherein determining  
2 whether the colonies have active glucose oxidase comprises checking for fluorescence.

1 9. A method for formulating an enzyme according to claim 2, wherein determining  
2 whether the colonies have peroxide resistant properties comprises:  
3 incubating the colonies in peroxide; and  
4 determining whether the colonies have active glucose oxidase after incubating the  
5 colonies in peroxide.

1 10. A method for formulating an enzyme according to claim 2, wherein testing  
2 glucose oxidase from the colonies for functionality comprises employing glucose oxidase from  
3 the colonies in sensors.

1 11. A method for formulating an enzyme according to claim 10, wherein employing  
2 glucose oxidase from the colonies in sensors comprises:  
3 extracting glucose oxidase from the colonies;

4 immobilizing the glucose oxidase after extracting the glucose oxidase from the  
5 colonies;  
6 placing the immobilized glucose oxidase in a sensor; and  
7 testing the sensor.

1 12. A method for formulating an enzyme according to claim 11, wherein extracting  
2 glucose oxidase from the colonies comprises employing an ionic column to extract glucose  
3 oxidase from the colonies.

1 13. A method for formulating an enzyme according to claim 11, wherein extracting  
2 glucose oxidase from the colonies comprises:  
3 removing the glucose oxidase from the colonies;  
4 purifying the glucose oxidase; and  
5 characterizing the glucose oxidase.

1 14. A method for formulating an enzyme according to claim 13, wherein removing  
2 the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell  
3 components.

1 15. A method for formulating an enzyme according to claim 14, wherein removing  
2 the glucose oxidase from the colonies further comprises fractionating the cell components  
3 employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

1 16. A method for formulating an enzyme according to claim 13, wherein removing  
2 the glucose oxidase from the colonies comprises disrupting the colonies into cell components via  
3 sonication.

1 17. A method for formulating an enzyme according to claim 16, wherein removing  
2 the glucose oxidase from the colonies further comprises fractionating the cell components  
3 employing centrifugation and differential solubility after disrupting the colonies via sonication.

1 18. A method for formulating an enzyme according to claim 13, wherein purifying  
2 the glucose oxidase comprises purifying the glucose oxidase by employing chromatography  
3 methods.

1 19. A method for formulating an enzyme according to claim 1, wherein the glucose  
2 oxidase is obtained from an organism and wherein the organism is selected from a group  
3 consisting of *Aspergillus Niger*, *Penicillium funiculosum*, *Saccharomyces cerevisiae*, and  
4 *Escherichia Coli*.

1 20. A method for formulating an enzyme according to claim 1, wherein creating at  
2 least one mutated glucose oxidase gene comprises employing polymerase chain reaction  
3 techniques to create at least one mutated glucose oxidase gene.

1 21. A method for formulating an enzyme according to claim 1, wherein creating at  
2 least one mutated glucose oxidase gene comprises employing error-prone polymerase chain  
3 reaction techniques to create at least one mutated glucose oxidase gene.

1 22. A method for formulating an enzyme according to claim 1, wherein creating at  
2 least one mutated glucose oxidase gene comprises employing gene shuffling techniques to create  
3 at least one mutated glucose oxidase gene.

1 23. A method for formulating an enzyme according to claim 1, wherein the method  
2 further comprises creating a next generation of mutated glucose oxidase genes after screening the  
3 colonies for desirable properties.

1 24. A method for formulating an enzyme according to claim 23, wherein creating a  
2 next generation of mutated glucose oxidase genes is repeated approximately 2 to 6 times.

1 25. An enzyme formulated according to the method of claim 1.

1 26. A method for formulating an enzyme comprising:  
2 obtaining an organism with a glucose oxidase gene;  
3 growing multiple colonies of the organism;  
4 altering the environment of the colonies; and  
5 screening the colonies to identify colonies with active glucose oxidase after  
6 altering the environment of the colonies.

1 27. A method for formulating an enzyme according to claim 26, wherein the  
2 organism is selected from a group consisting of *Aspergillus Niger*, *Penecillium funiculosum*,  
3 *Saccharomyces cerevisiae*, and *Escherichia Coli*.

1 28. A method for formulating an enzyme according to claim 26, wherein altering the  
2 environment of the colonies comprises introducing peroxide to the colonies.

1           29.     A method for formulating an enzyme according to claim 26, wherein screening  
2     the colonies to identify colonies with active glucose oxidase comprises employing a substance  
3     that changes color in the presence of active glucose oxidase.

1           30.     A method for formulating an enzyme according to claim 29, wherein the  
2     substance is leuco-crystal-violet.

1           31.     A method for formulating an enzyme according to claim 30, wherein screening  
2     the colonies to identify colonies with active glucose oxidase comprises checking for  
3     fluorescence.

1           32.     A method for formulating an enzyme according to claim 26, wherein the method  
2     further comprises testing the colonies with active glucose oxidase for functionality after  
3     screening the colonies to identify colonies with active glucose oxidase.

1           33.     A method for formulating an enzyme according to claim 32, wherein the method  
2     further comprises continuing to alter the environments of the colonies until the colonies with  
3     active glucose oxidase are of a suitable number to proceed with testing the colonies with active  
4     glucose oxidase for functionality.

1           34.     A method for formulating an enzyme according to claim 32, wherein testing the  
2     colonies with active glucose oxidase for functionality comprises employing glucose oxidase  
3     from the colonies in sensors.

1           35.     A method for formulating an enzyme according to claim 32, wherein testing the  
2     colonies with active glucose oxidase for functionality comprises:

3 extracting glucose oxidase from the colonies;  
4 immobilizing the glucose oxidase after extracting the glucose oxidase from the  
5 colonies;  
6 placing the immobilized glucose oxidase in a sensor; and  
7 testing the sensor.

1 36. A method for formulating an enzyme according to claim 35, wherein extracting  
2 glucose oxidase from the colonies comprises employing an ionic column to extract glucose  
3 oxidase from the colonies.

1 37. A method for formulating an enzyme according to claim 35, wherein extracting  
2 glucose oxidase from the colonies comprises:  
3 removing the glucose oxidase from the colonies;  
4 purifying the glucose oxidase; and  
5 characterizing the glucose oxidase.

1 38. A method for formulating an enzyme according to claim 37, wherein removing  
2 the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell  
3 components.

1 39. A method for formulating an enzyme according to claim 38, wherein removing  
2 the glucose oxidase from the colonies further comprises fractionating the cell components  
3 employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

1           40.    A method for formulating an enzyme according to claim 37, wherein removing  
2   the glucose oxidase from the colonies comprises disrupting the colonies into cell components via  
3   sonication.

1           41.    A method for formulating an enzyme according to claim 40, wherein removing  
2   the glucose oxidase from the colonies further comprises fractionating the cell components  
3   employing centrifugation and differential solubility after disrupting the colonies via sonication.

1           42.    A method for formulating an enzyme according to claim 37, wherein purifying  
2   the glucose oxidase comprises purifying the glucose oxidase by employing chromatography  
3   methods.

1           43.    An enzyme formulated according to the method of claim 26.

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